Hill coefficients CAF 2.14 ± 0.16, HF 2.53 ± 0.14, P<0.05). Consistently, the results of real time PCR and western blot demonstrated that captopril significantly downregulated the expression of apamin sensitive SK channels (SK3 mRNA: CAF 2.10 ± 0.9, n = 6 vs HF 8.40 ± 2.10, n = 6; SK3 protein: CAF 0.40 ± 0.07, n = 6 vs HF 0.56 ± 0.09, n = 6).

CONCLUSIONS Captopril significantly downregulated the sensitivity of SK channels to $[Ca^{2+}]i$ and the SK3 channels expression in HF, and reversed the SK channels remodeling.

GW26-e1586

AMPK attenuates proliferation of cardiac fibroblast via regulating TGF-β1/Smad pathways

Yili Chen,[1] Chunxi Lin,[1] Yufang Zhong,[2] Guiying Lu,[1] Yuedong Ma,[1] Huiling Huang[1]
[1]Department of Cardiology, the First Affiliated hospital of Sun Yat-Sen University; [2]Department of Nephrology, the First Affiliated hospital of Sun Yat-Sen University

OBJECTIVES AMP-activated protein kinase (AMPK) exerts inhibitory effects on cardiac hypertrophy. However, the mechanism remains unclear. The aim of the present study was to investigate the effects of AMPK on angiotensin II (AngII) -induced proliferation of cardiac fibroblast and the mechanisms involved.

METHODS Proliferation of cardiac fibroblast was induced by angiotensin II (AngII). Cardiac fibroblasts were treated with the specific AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR, 0.5 mmol/L) and the specific AMPK antagonist Compound C (1 umol/L), and then stimulated with AngII (1 umol/L). Cell proliferation and the DNA synthesis were measured by MTT assay and EdU incorporation assay. TGF-β1 and Smad2, 3, 4 mRNA and protein expression was detected using Real-Time PCR and western blot analysis.

RESULTS Activation of AMPK by AICAR could inhibit AngII-induced proliferation of cardiac fibroblasts, manifesting decreased DNA synthesis and collagen production ($P<0.05$). Moreover, AngII significantly increased the mRNA and protein expression of TGF-β1 and Smad2, 3, 4 ($P<0.05$). AMPK activation markedly reversed the elevated TGF-β1 and Smad2, 3, 4 mRNA and protein levels ($P<0.05$). Furthermore, Treatment of proliferated cardiac fibroblasts with Compound C blunted the effects of AMPK on proliferation of cardiac fibroblasts and changes to the TGF-β1/Smad pathway ($P<0.05$).

CONCLUSIONS AMPK activation could attenuate proliferation of cardiac fibroblast induced by AngII, which may be due to the inhibition of TGF-β1/Smad pathways.

GW26-e4768

Improved Recovery After Myocardial Ischemic Infarction by Copper Supplementation

Pengfei Han,[1] Xiang Zhou,[1] Wenjing Zhang,[1] Qipu Feng,[1] Ying Xiao,[1] Xiaorong Sun,[1] Xinmei Zhao,[1] Haiying Fu,[1] Y. James Kang[1,2]
[1]Regenerative Medicine Research Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China; [2]Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky, USA

OBJECTIVES Depressed angiogenesis due to ischemic injury leads to myocardial infarction. Copper (Cu) is involved in angiogenesis and ischemia causes copper loss in the heart. The present study was undertaken to test the hypothesis that Cu supplementation improves myocardial angiogenesis, leading to regression of myocardial ischemic infarction in Rhesus monkey model.

METHODS Coronary artery ligation was used to produce myocardial ischemia and the monkeys developed myocardial infarction 4 weeks after ischemia. A newly developed ultrasound contrast microbubble composed of Cu-albumin coated structure was used to specifically deliver Cu into the infarct area. The treatment was performed twice a week for 4 weeks.

RESULTS This procedure effectively increased Cu concentrations in the infarct area and activated the angiogenesis factors including vascular endothelial growth factor (VEGF), VEGF receptor-1 (VEGFR-1), and other relevant factors. Along with these changes, myocardial infarct size was significantly decreased and the density of myocardial microvessels was significantly increased. In addition, cardiac function was significantly recovered, as evidenced by increased ejection fraction (EF) values and decreased end-systolic volume (ESV) measured by echocardiography.

CONCLUSIONS This study thus demonstrated that Cu supplementation improved cardiac structural and functional recovery after ischemic infarction.

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GW26-e5402

Discovery of a new conduction substrate associated with atrioventricular node-anterior extension pathway

Fan Wang, Shuo Zhang
Second Affiliated Hospital of Harbin Medical University

OBJECTIVES The atrioventricular node(AVN) plays a role in conducting action potentials at an appropriate conduction velocity from atria to ventricles. Its complex anatomical structures and functional longitudinal dissociations are considered important in delayed conduction and AVN reentrant tachycardia (AVNRT). Inferior nodal extensions(INE) are part of the AVN. It is thought that these extensions may be involved in slow-pathway conduction and are part of the underlying circuitry that causes AVNRT. Some other conduction tissue shared the same origin layers with AVN distribute around the two valve annulus. The retroaortic node defined as the enlargement of this node-like tissue is located on the right side of the atrium parallel with the aorta. The potential electrophysiological function of these node-like tissues is still unknown. Understanding their detail anatomical structure, histological features and electrophysiological behavior are significant to clear the complex conduction characteristics of the AV junction.

METHODS Adult rats(n=6), mice(n=5) and rabbit(n=5) were used. Serial sections from the entire AV junction were obtained. Masson's trichrome stain was performed on AVN regions to assess for fibrous tissue. Three connexins(Cx) proteins including Cx43, Cx40 and Cx45 which dominate the electronic conduction of the heart and three main ion channels($Na_v1.5$, $Ca_v3.1$ and HCN4) participating the depolarization of myocardial cell were immunohistochemically labeled. Serial sections were used to reconstruct a 3D computational model of the anatomy of the AV junction which display the different histological features at different levels.

RESULTS There appears to be an anterior extension of the AVN which connect retroaortic node and AVN. The anterior node extension (ANE), compact node and INE express the same connexin isoforms. $Na_v1.5$ labeling was abundant in the atrial and ventricular myocardium. $Na_v1.5$ labeling presents at a reduced level in the compact node, ANE and INE. $Ca_v3.1$ and HCN4 expression were mainly expressed in the $Na_v1.5$ reduced area. Further, connections between the atria and inferior extension occur indirectly via small branches. However, this was distinct from the connection pattern that we observed between the atria and ANE, which was direct.

CONCLUSIONS We conclude that the retroaortic node connect with ANE forming ANE which suggests there would be direct electric conduction between them. Characteristics of these structures are conserved among various species including rat, mouse and rabbit as we had proven. ANE, AVN and INE have nearly the same electronic level and action potential level structure basic that highlight they would have the same conduction properties, but there are different connection patterns in atrium between them that suggests there would be the subtle conduction velocity difference after accept the impulse, which provide a new location and substrate of conduction that may present new insights on mechanisms underlying normal AV conduction and AVNRT.

GW26-e2274

AVE 0991, Nonpeptide angiotensin-(1-7) analogue, modulates cardiac hypertrophy via reducing oxidative stress

Yili Chen,[1] Yufang Zhong,[2] Chunxi Lin,[1] Guiying Lu,[1] Huiling Huang,[1] Yuedong Ma[1]
[1]Department of Cardiology, the First Affiliated hospital of Sun Yat-Sen University; [2]Department of Nephrology, the First Affiliated hospital of Sun Yat-Sen University

OBJECTIVES AVE 0991, the nonpeptide angiotensin-(1-7) (Ang-(1-7)) analog, is recognized as having beneficial cardiovascular effects. However, the mechanisms have not been fully elucidated. This study was designed to investigate the effects of AVE 0991 on cardiac hypertrophy and the mechanisms involved.

METHODS Mice were subjected to aortic banding (AB) to induce cardiac hypertrophy. *After treatment with* AVE 0991 (20 mg·kg·day (-1)) for four weeks, indices of cardiac hypertrophy and heart function were measured by echocardiography, histological analyses and quantitative

real-time PCR (q-PCR). NADPH oxidase (NOX) 2 and NOX4 mRNA and protein expression of left ventricular tissues was detected using q-PCR and western blot analysis that are associated with oxidative stress.

RESULTS AVE 0991 displayed a significant reduction in the left ventricular weight (15.96±0.68 vs. 22.21±0.75, $P < 0.01$) and left ventricular end-diastolic diameter (3.48±0.19 vs. 4.32±0.20, $P < 0.05$), and a significant elevation in left ventricular ejection fraction (58.16±2.78 vs. 41.82±5.58, $P < 0.05$) when compared to the vehicle-treated AB group. Moreover, we found that the mean myocyte diameter (13.53± 0.56 vs. 15.46 ± 0.21, $P < 0.01$) and the gene expression of the hypertrophic markers atrial natriuretic peptide (ANP) ($P < 0.01$) and β-MHC ($P < 0.01$) were markedly decreased in the AVE0991 group. Furthermore, AVE 0991 inhibited the mRNA and protein expression of NOX 2 ($P < 0.01$) and NOX 4 ($P < 0.01$) when compared to the vehicle-treated AB group.

CONCLUSIONS Our data showed that AVE 0991 treatment could attenuate cardiac hypertrophy and improve heart function, which may be attributed to reducing the oxidative stress.

GW26-e2369

Nicotine Exposure Causes GATA4 and Tbx5 Gene Repression by DNA Hypermethylation during Cardiac Myogenesis

Xueyan Jiang,[1,2] Xiaohong Li,[1] Juan Feng,[1] Qinxiong Lin,[1] Ming Yang,[1] Yangxin Li,[2] Yong-Jian Geng,[1] Mengzhen Zhang,[1] Liping Mai,[1] Xiyong Yu[1]
[1]Medical Research Center of Guangdong General Hospital, Guangdong Cardiovascular Institute, Guangdong Academy of Medical Sciences; [2]University of Texas Medical School at Houston and Texas Heart Institute, Houston, Texas 77030, USA

OBJECTIVES Maternal nicotine exposure caused alteration of gene expression patterns and programming of cardiovascular dysfunction. This study was to investigate effect of nicotine on cardiac gene expression and epigenetic regulation during cardiac myogenesis.

METHODS To study effect of nicotine on cardiac myogenesis, in vitro and in vivo cardiac developmental model were established respectively. Mouse embryonic bodies (EBs) derived from mouse embryonic stem cells were induced to 12-day cardiac differentiation with or without nicotine treatment. As in vivo cardiac myogenic model, pregnant Sprague-Dawley rats were exposed to nicotine through gestation, hearts were isolated from neonatal offspring for further molecular study after echocardiography for heart function.

RESULTS In vitro study shows nicotine exposure selectively inhibited expression of two cardiac genes (GATA4 and *Tbx5*) in both mRNA and protein expression level. Persistent nicotine exposure resulted in up-regulation of 5-methylcytosine, DNMT1 and DNMT3A but decreased GATA4 and Tbx5 gene expression due to promoter DNA hypermethylation. However, no significant effect has been found on mESCs proliferation and two embryonic biomarkers'(Oct4 and Nanog) mRNA expression with nicotine treatment. Nicotine exposure also decreased amounts of beating EBs and reduced GATA4 positive cells at 12-day EBs. This nicotine-induced suppression was reversed by general nicotinic acetylcholine receptors (nAChRs) inhibitor, suggesting the involvement of nAChRs in the direct adverse impact of nicotine on cardiac differentiation. Consistent results of GATA4 and Tbx5 gene suppression and promoter DNA hypermethylation by maternal nicotine treatment were obtained from in vivo cardiac development model. Echocardiography showed impaired cardiac function in offspring including reduced ejection fraction (EF%), systolic and diastolic left ventricular anterolateral wall (LVAW;s and LVAW;d) as well as systolic and diastolic left ventricular posterior wall (LVPW;s and LVPW;d).

CONCLUSIONS This study presents a direct repressive effect of nicotine on cardiac transcriptional factors (GATA4 and Tbx5) by promoter DNA hypermethylation during cardiac myogenesis. Reduction of spontaneous beating EBs and impaired cardiac function in offspring heart has been found with nicotine exposure.

GW26-e3983

Ca2+/calmodulin-dependent protein kinase modulation of torsade de pointes arrhythmogenesis and identification of targeted sites of antiarrhythmic therapy in human Timothy Syndrome arising from a new CACNA1C mutation

Jieyun Bai,[1] Kuanquan Wang,[1] Henggui Zhang[1,2]
[1]School of Computer Science and Technology, Harbin Institute Technology, Harbin, China; [2]School of Physics and Astronomy, University of Manchester, Manchester, UK

OBJECTIVES Timothy syndrome (TS) is a malignant form of congenital long QT syndrome with excessive cellular Ca^{2+} entry and torsade de pointes (TdP) arrhythmias often triggered by a variety of neurohormonal and second-massager pathways. We sought to explore mechanisms by which Ca^{2+}/ calmodulin-dependent protein kinase (CaMKII) modulates arrhythmogenesis and to identify potential targeted sites of antiarrhythmic therapy in TS arising from a novel mutation (CACNA1C, p.G1911R).

METHODS A 15 mm×15 mm two-dimensional (2D) multicellular transmural tissue model was developed by integrating an anatomically ventricular geometry of the human ventricular tissue sheet and a dynamic human ventricular myocyte model incorporated with a detailed CaMKII module in the format of mono-domain model. To better understand the TS, L-type Ca^{2+} current (I_{CaL}) equations of the myocyte model were modified based on experimental conditions (current density increased ~20%, $V_{1/2}$ of activation shifted ~-5mV, $V_{1/2}$ of inactivation shifted ~+6 mV, tau of inactivation increased ~20%). To explore ionic mechanisms of CaMKII-dependent TdP, proarrhythmic substrates were compared and analyzed. In addition, in order to investigate mechanisms initiating and maintaining TdP, the spatial organization of repolarization and arrhythmogenesis were determined in the 2D transmural tissue model.

RESULTS TS ventricular myocytes exhibited more activated CaMKII(~50%), increased I_{CaL} facilitation (~55%), higher peak Ca^{2+} transient (~83%), augmented frequency of Ca^{2+} sparks (~200%), enhanced maximum SR Ca^{2+} content (~34%), prolonged action potential duration (APD) and afterdepolarizations. On the one hand, CaMKII-dependent SR overload resulted in SR Ca2+ leak for triggering delayed afterdepolarizations (DADs); on the other hand, CaMKII-dependent I_{CaL} facilitation contributed to excessive action potential prolongation in midmyocardial (M) cells (from 413.6 to 1133.9 ms) which favors the generation of early afterdepolarizations(EADs). The excessive prolongation of APD in the M cells caused an abrupt rise in transmural dispersion of repolarization (from 33.06 ms/mm to 52.99 ms/mm) and M cells formed zones of increased refractoriness, producing steep spatial gradients of repolarization that were directly responsible for conduction block and self-sustained intramural reentrant circuits underlying TdP. However, CaMKII inhibition reversed an increase in intracellular Ca2+, normalized action potential and prevented TdP.

CONCLUSIONS These computer simulations suggest that TS-mediated Ca^{2+} influx is an upstream initiating event for arrhythmia phenotypes that are ultimately dependent on CaMKII activation, the M region of TS can increase intrinsic heterogeneities of cardiac tissue and resulte in the generation and maintenance of reentrant excitations underlying TdP, and CaMKII blockers may provide additional antiarrhythmic effect in patients with TS.

GW26-e4017

Intravenous infusion of drag-reducing polymers protects against acute myocardial ischemia and reperfusion injury

Feng Hu,[1] Daogang Zha,[2] Yili Liu,[2] Kaizheng Gong,[1] Gaoyuan Ge,[1] Pei Zhao,[1] Lin Du,[1] Xiaoning Sun,[1] Zhengang Zhang[1]
[1]Department of Cardiology, the Second Clinical Medical School of Yangzhou University, Yangzhou, China; [2]Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou, China

OBJECTIVES Drag-reducing polymers (DRPs) are blood-soluble macromolecules that can increase blood flow and reduce vascular resistance. It has been widely used in petroleum transportation, irrigation, navigation and other industrial pipeline. In recent years, the potential medical application of DRPs had been explored in cardiovascular disease, atherosclerosis, shock and other fields. The purpose of the present study is to observe the effect of DRPs on myocardial ischemia/ reperfusion (I/R) injury in rat model.

METHODS Adult Wistar rats were randomly divided into three groups (n=16): DRP group, Control group and Sham group. Acute myocardial infarction achieved by occluding left anterior descending coronary artery (LAD). After 30 min of ischemia, the LAD was released 120 min to induce I/R injury. Sham animals underwent left thoracotomy only. Rats in DRP group were injected with 5×10^{-5} g/ml DRP solution through the right jugular vein at a constant rate of 3.5 ml/h for 30 min during reperfusion. Saline was administered in control group and sham group. Ejection fraction was measured by echocardiography after 120 min reperfusion. A catheter inserted into left ventricle to measure left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP). Myocardial infarct size were also been measured.

RESULTS All rats in sham group survived through 150 min observation period, the survival rate in DRP group was 81.25% (13/16),